ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 2005, p. 5133–5135 Vol. 49, No. 12
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.12.5133–5135.2005


# Efficacy of Caspofungin against *Aspergillus terreus*

Francesco Barchiesi,[1]* Elisabetta Spreghini,[1] Alfredo Santinelli,[2] Annette W. Fothergill,[6] Stefania Fallani,[5] Esther Manso,[4] Eleonora Pisa,[2] Daniele Giannini,[3] Andrea Novelli,[5] Maria I. Cassetta,[5] Teresita Mazzei,[5] Michael G. Rinaldi,[6,7] and Giorgio Scalise[1]

*Istituto di Malattie Infettive e Medicina Pubblica,[1] Istituto di Anatomia Patologica,[2] and Centro Gestione Presidenza Medicina e Chirurgia, Università Politecnica delle Marche,[3] and Laboratorio di Microbiologia Azienda Ospedaliero-Universitaria Ospedali Riuniti Umberto I-Lancisi-Salesi, Ancona, Italy[4]; Istituto di Farmacologia Preclinica e Clinica, Università degli Studi di Firenze, Florence, Italy[5]; Department of Pathology, The University of Texas Health Science Center at San Antonio, San Antonio, Texas[6]; and Audie L. Murphy Division, South Texas Veterans Health Care System, San Antonio, Texas[7]*

Received 23 July 2005/Returned for modification 1 September 2005/Accepted 3 October 2005

**We investigated the in vitro and in vivo activities of caspofungin against *Aspergillus terreus*. The drug increased survival and reduced tissue fungal burden in neutropenic mice. Therefore, our data support the role of caspofungin in treating systemic infections due to this emerging pathogen.**

Invasive aspergillosis (IA) has emerged as a common cause of morbidity and mortality among immunocompromised patients (2, 6). The frequency of IA caused by *A. terreus* is increasing (5, 10–13), and an optimal therapy for this emerging mold infection remains to be clarified (10–13).

Caspofungin (CAS) was shown to be effective for the treatment of IA in patients refractory to or intolerant of amphotericin B (AMB) (7). Here, we investigated the efficacy of CAS against *A. terreus*.

Two clinical isolates of *A. terreus* (*A. terreus* 1 and *A. terreus* 2) were utilized in this study (Table 1). CAS was used as a commercial preparation (Cancidas) dissolved in sterile distilled water and in sterile saline for in vitro and in vivo studies, respectively. AMB was used as pure powder (Sigma) dissolved in dimethyl sulfoxide for in vitro studies and as a commercial preparation (Fungizone) dissolved in sterile saline for in vivo studies. Stock solution of itraconazole (ITC; Janssen) was prepared in polyethylene glycol 200 for both in vitro and in vivo studies.

To investigate the in vitro activity of three antifungal agents, a broth microdilution method was performed according to the Clinical and Laboratory Standards Institute (8). Final concentrations of each drug ranged from 0.03 to 16 μg/ml. AMB and ITC MICs were considered the lowest concentrations of antifungal compound that yielded no growth. CAS activity was analyzed by considering the minimum effective concentration (MEC) instead of the MIC (1). The minimum fungicidal concentration (MFC) of each drug was determined by culturing 100 μl of broth from all the wells above the MIC (or MEC) onto Sabouraud dextrose agar plates. The MFC was defined as the lowest concentration of antifungal compound yielding no growth. Experiments were conducted in quintuplicate.

CD1 male mice (25 g; Charles River Laboratories, Calco, Italy) were utilized in all in vivo studies. Mice were rendered neutropenic by intraperitoneal (i.p.) administration of cyclophosphamide (200 mg/kg of body weight) on days −4, +1, and +4 and every 3 days thereafter. Animal experiments were conducted with the approval of the University of Ancona ethics committee.

A murine model of systemic aspergillosis was established by intravenous injection of approximately $5 \times 10^5$ conidia of each *A. terreus* isolate. CAS was administered i.p. at doses of 0.5, 1, 2.5, and 5 mg/kg/day. AMB was administered i.p. at 2.5 mg/kg/day. ITC was administered by oral gavage at 30 and 100 mg/kg/day. The drug was given in two divided doses. All drugs were initiated 3 h postinfection.

In survival studies, the mice were treated daily from day 0 to day 9 (10 consecutive days) and observed through day 10 to day 20 postinfection. In tissue burden experiments, the mice were treated daily from day 0 to day 4 (5 consecutive days) and

TABLE 1. In vitro susceptibility of clinical isolates of *Aspergillus terreus* to caspofungin, amphotericin B, and itraconazole[a]

| Isolate | Drug[b] | Susceptibility results (μg/ml) reported as: | | | |
|---|---|---|---|---|---|
| | | MIC (or MEC[b]) | | MFC[c] | |
| | | Median | Range | Median | Range |
| *A. terreus* 1 | CAS | 0.5 | 0.25–1.0 | >16 | >16 |
| | AMB | 4.0 | 1.0–4.0 | >16 | >16 |
| | ITC | 0.125 | 0.06–0.25 | 8.0 | 4.0–16 |
| *A. terreus* 2 | CAS | 1.0 | 0.25–1.0 | >16 | >16 |
| | AMB | 2.0 | 2.0–4.0 | >16 | >16 |
| | ITC | 0.06 | 0.06–0.25 | >16 | 4.0–>16 |

[a] Each testing was run in quintuplicate and repeated on 2 different days.
[b] MEC was determined microscopically, and it was defined as the least concentration of CAS causing abnormal hyphal growth with short abundant branchings.
[c] MFC was defined as the lowest concentration of antifungal compound yielding no growth.

* Corresponding author. Mailing address: Istituto di Malattie Infettive e Medicina Pubblica, Università Politecnica delle Marche, Azienda Ospedaliero-Universitaria, Ospedali Riuniti Umberto I-Lancisi-Salesi, Via Conca, Torrette, Ancona 60020, Italy. Phone: 39.0715963467. Fax: 39.0715963468. E-mail: l.infettive@ao-umbertoprimo.marche.it.

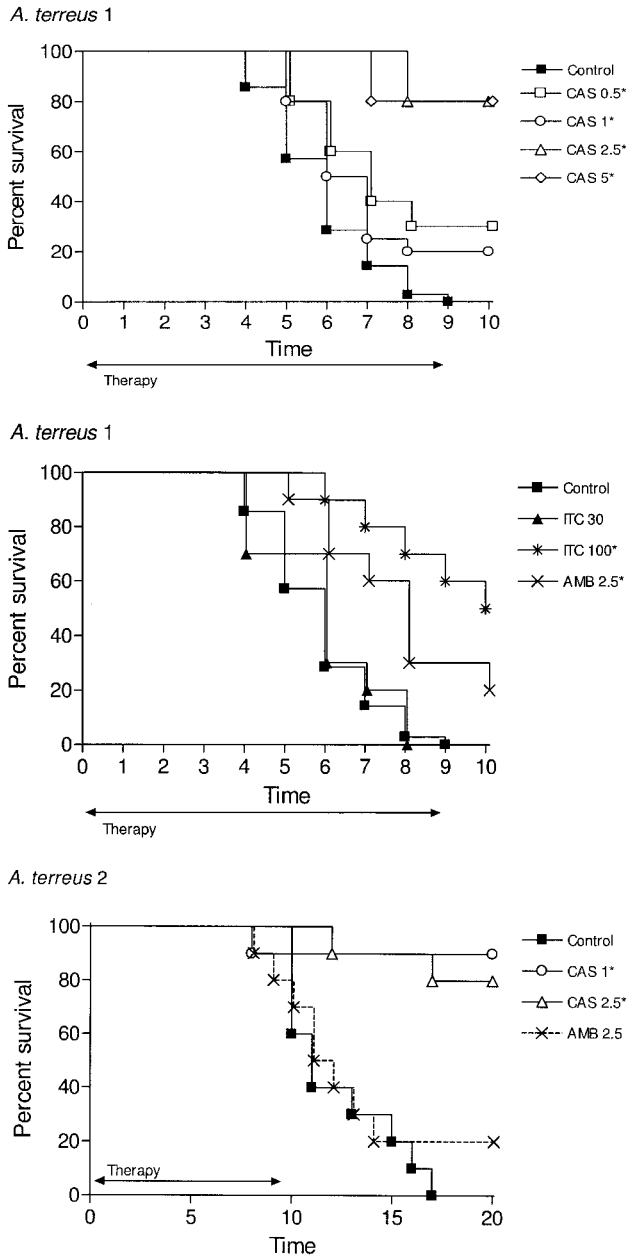


FIG. 1. Survival of mice infected intravenously with approximately $5 \times 10^5$ conidia per mouse of two strains of *A. terreus*. Both studies were conducted by initiating the therapy 3 h postinfection (day 0) and continued through day 9 postinfection (10 consecutive days). There were 10 mice in each group, with the exception of the control group and the group of mice treated with CAS at 1 mg/kg/day in studies with *A. terreus* 1 containing 35 and 20 mice, respectively. Asterisks indicate groups with prolonged survival over controls ($P$, $<0.05$).

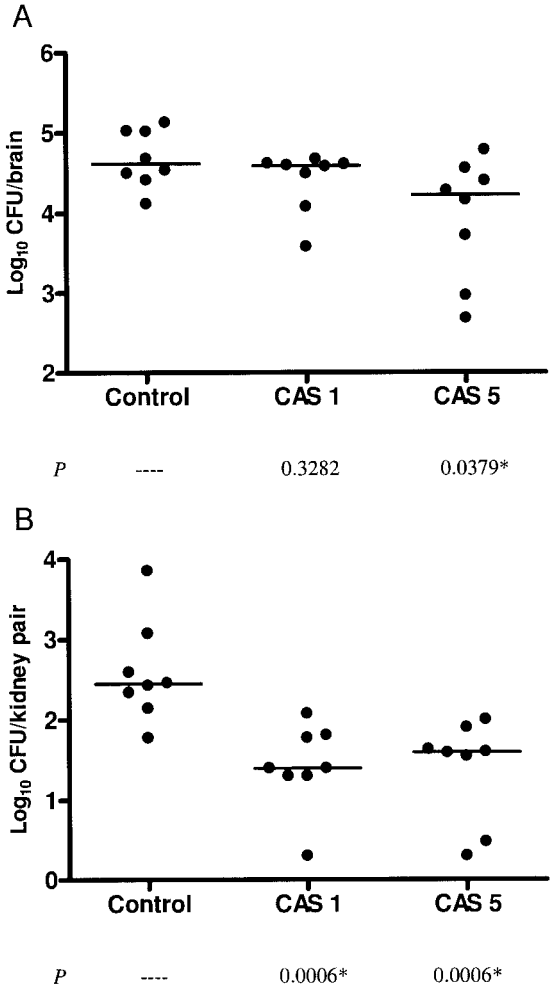


FIG. 2. Tissue burden of neutropenic CD1 mice. The mice were infected intravenously with approximately $5 \times 10^5$ conidia of *A. terreus* 1 and treated with CAS administrated i.p. at doses of 1 and 5 mg/kg/day. The therapy was initiated 3 h postinfection and administrated daily from day 0 to day 4 (5 consecutive days), and the mice were sacrificed on day 5 postinfection (24 h after the last dose). Total brain (A) and kidney (B) tissue fungal burden was expressed as $\log_{10}$ of total CFU. The bars represent the medians. Asterisks indicate groups with tissue burden counts lower than control ($P$, $<0.05$).

sacrificed on day 5 postinfection (24 h after the last dose). Brain and both kidneys from each animal were aseptically removed, homogenized, and plated onto Sabouraud dextrose agar plates for colony count determination.

CAS plasma levels were determined after 1 day and 5 consecutive days of CAS therapy at 1 and 5 mg/kg/day, respectively. CAS concentrations were determined by high-performance liquid chromatography (lowest sensitivity limit, 0.125 mg/liter) with a Perkin-Elmer Series 200 high-performance liquid chromatograph (9).

The Mann-Whitney U test was performed to analyze both in vitro and tissue burden data. The survivals studies were analyzed by log rank and plotted by Kaplan-Meier curves. All $P$ values of $<0.05$ were considered significant.

CAS MECs (range, 0.25 to 1.0 μg/ml) were significantly lower than AMB MICs (range, 2.0 to 4.0 μg/ml; $P$, $<0.0001$) but significantly higher than ITC MICs (range, 0.06 to 0.25 μg/ml; $P$, 0.0001) (Table 1). Therefore, the approximate rank order of antifungal activity determined by MIC (or MEC) (from most to least activity) was ITC, CAS, and AMB. Both CAS and AMB MFCs were >16 μg/ml, while ITC MFCs ranged from 4.0 to >16 μg/ml. No statistical differences were observed among MFCs of the three drugs.

In mice infected with *A. terreus* 1, all doses of CAS signifi-

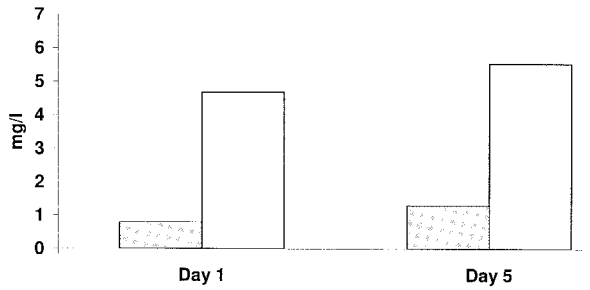


FIG. 3. CAS plasma levels. Plasma sampling was performed after 1 day and 5 consecutive days of CAS therapy at 1 mg/kg/day (gray column) and 5 mg/kg/day (white column). Blood samples were drawn at 2 h postdose. Values represent the mean concentrations plus or minus standard deviations of seven mice per group.

cantly prolonged the survival against the controls (Fig. 1). Similarly, AMB and ITC at 100 mg/kg/day (ITC100), but not ITC30, were effective. In mice challenged with *A. terreus* 2, both CAS1 and CAS2.5, but not AMB, prolonged significantly the survival against the controls (Fig. 1).

Tissue burden studies were conducted with only *A. terreus* 1, and the results are shown in Fig. 2. Mice were treated daily for 5 consecutive days with CAS given at 1 and 5 mg/kg/day and sacrificed 1 day after the end of treatment for colony count determination in the brain and kidney tissues. CAS5, but not CAS1, was effective at reducing the CFU against the controls in the brain tissue. On the other hand, both CAS1 and CAS5 were effective in kidneys. Finally, CAS plasma levels measured by high-performance liquid chromatography increased over time from (medians plus or minus standard deviations) 0.79 ± 0.03 to 1.30 ± 0.02 mg/liter in mice treated with CAS1 and from 4.68 ± 0.58 to 5.54 ± 0.40 mg/liter in mice treated with CAS5 (Fig. 3).

In terms of MIC (or MEC), CAS was superior to AMB but less effective than ITC. The few in vitro data of CAS against *A. terreus* that are available so far reveal a wide variation of results depending on the end point definition (i.e., MIC versus MEC). Our MEC data (range, 0.25 to 1.0 μg/ml) are similar to those reported by Arikan et al. (1).

Our in vivo findings confirmed and extended those recently reported by Graybill et al. (3). They found that the echinocandin compound prolonged the survival at doses as low as 0.5 mg/kg/day, while only higher doses (i.e., CAS at 10 mg/kg/day) were effective at reducing the fungal burden in the spleen but not in the lung tissues (3). In our experimental model, all doses of CAS (range, 0.5 to 5 mg/kg/day) were effective at prolonging the survival. In addition, CAS given at either 1 or 5 mg/kg/day was effective at reducing the fungal burden in kidney. It has been reported that CAS concentrations in this organ are usually three times higher than those found in plasma (4). Since we found median plasma concentrations of 1.3 and 5.5 mg/liter after 5 consecutive days of CAS given at 1 and 5 mg/kg/day, respectively, one can speculate that both doses were able to keep tissue concentrations several times higher than the MECs reported for this isolate (range, 0.25 to 1.0 μg/ml). The finding that only the highest dose of CAS was effective in the brain is easily explained by the fact that the brain/plasma ratio is 0.06 (4) and that only CAS given at 5 mg/kg/day would have reached tissue concentrations close to the MECs.

In conclusion, our study reinforces the role of this drug in infections due to this emerging pathogen.

## REFERENCES

1. **Arikan, S., M. Lozano-Chiu, V. Paetznick, and J. H. Rex.** 2001. In vitro susceptibility testing methods for caspofungin against *Aspergillus* and *Fusarium* isolates. Antimicrob. Agents Chemother. **45:**327–330.
2. **Denning, D.** 1998. Invasive aspergillosis. Clin. Infect. Dis. **26:**781–785.
3. **Graybill, J. R., S. Hernandez, R. Bocanegra, and L. K. Najvar.** 2004. Antifungal therapy of murine *Aspergillus terreus* infection. Antimicrob. Agents Chemother. **48:**3715–3719.
4. **Hajdu, R., R. Thompson, J. G. Sundelof, B. A. Pelak, F. A. Bouffard, J. F. Dropinski, and H. Kropp.** 1997. Preliminary animal pharmacokinetics of the parenteral antifungal agent MK-0991 (L-743,872). Antimicrob. Agents Chemother. **41:**2339–2344.
5. **Iwen, P., M. Rupp, A. Langnas, E. Reed, and S. Hinrichs.** 1998. Invasive pulmonary aspergillosis due to *Aspergillus terreus*: 12-year experience and review of the literature. Clin. Infect. Dis. **26:**1092–1097.
6. **Latgé, J.-P.** 1999. *Aspergillus fumigatus* and aspergillosis. Clin. Microbiol. Rev. **12:**310–350.
7. **Maertens, J., I. Raad, G. Petrikkos, M. Boogaerts, D. Selleslag, F. B. Petersen, C. A. Sable, N. A. Kartsonis, A. Ngai, A. Taylor, T. F. Patterson, D. W. Denning, T. J. Walsh, and the Caspofungin Salvage Aspergillosis Study Group.** 2004. Efficacy and safety of caspofungin for treatment of invasive aspergillosis in patients refractory to or intolerant of conventional antifungal therapy. Clin. Infect. Dis. **39:**1563–1571.
8. **National Committee for Clinical Laboratory Standards.** 2002. Reference method for broth dilution antifungal susceptibility testing of conidium-forming filamentous fungi. Approved standard M38-A. National Committee for Clinical Laboratory, Wayne, Pa.
9. **Schwartz, M., W. Kline, and B. Matuszewski.** 1997. Determination of a cyclic hexapeptide (L-743 872), a novel pneumocandin antifungal agent in human plasma and urine, by high-performance liquid chromatography with fluorescence detection. Analytica Chimica Acta **352:**299–307.
10. **Steinbach, W. J., D. K. Benjamin, Jr., D. P. Kontoyiannis, J. R. Perfect, I. Lutsar, K. A. Marr, M. S. Lionakis, H. A. Torres, H. Jafri, and T. J. Walsh.** 2004. Infections due to *Aspergillus terreus*: multicenter retrospective analysis of 80 cases. Clin. Infect. Dis. **39:**192–199.
11. **Steinbach, W. J., J. R. Perfect, W. A. Schell, T. J. Walsh, and D. K. Benjamin, Jr.** 2004. In vitro analyses, animal models, and 60 clinical cases of invasive *Aspergillus terreus* infection. Antimicrob. Agents Chemother. **48:** 3217–3225.
12. **Sutton, D. A., S. E. Sanche, S. G. Revankar, A. W. Fothergill, and M. G. Rinaldi.** 1999. In vitro amphotericin B resistance in clinical isolates of *Aspergillus terreus*, with a head-to-head comparison to voriconazole. J. Clin. Microbiol. **37:**2343–2345.
13. **Walsh, T. J., V. Petraitis, R. Petraitiene, A. Field-Ridley, D. Sutton, M. Ghannoum, T. Sein, R. Schaufele, J. Peter, J. Bacher, H. Casler, D. Armstrong, A. Espinel-Ingroff, M. G. Rinaldi, and C. A. Lyman.** 2003. Experimental pulmonary aspergillosis due to *Aspergillus terreus*: pathogenesis and treatment of an emerging fungal pathogen resistant to amphotericin B. J. Infect. Dis. **188:**305–319.